Docket No.: 60126-002US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Ayoub RASHTCHIAN, et al.	)	Confirmation No: 6375
Application No.: 10/633,629	)	Group Art Unit: 1633
Filed: August 5, 2004	)	Examiner: Ileana Popa

For: COMPOSITIONS FOR IN VITRO AMPLIFICATION OF NUCLEIC ACIDS

United States Patent and Trademark Office
Randolph Building
401 Dulany Street
Alexandria, Virginia 22314

Declaration under 37 C.F.R. § 1.132

I, Mr. Mark Berninger, declare and say:

1. I have received an Artium Baccalaureatus degree in Biology and pursued graduate studies at the Massachusetts Institute of Technology in cell and molecular biology, and have worked in the field of nucleic acid diagnostics and amplification for more than 15 years. I am not an employee of or affiliated with Quanta Biosciences, the assignee of the present application, but I am being compensated by Quanta Biosciences for the time spent in preparing this Declaration. I have extensive experience in the area of nucleic acid amplification, including the polymerase chain reaction. As director of a research group at Life Technologies Inc., I supervised experimental work on two novel nucleic acids amplification methods, both patented, and I am co-inventor of both. I am also co-inventor of a method to decontaminate PCR through the use of the enzyme uracil DNA glycosidase (UDG), which has been widely used and licensed. I also lead the development of products for carrying out PCR to amplify long regions of DNA and to assemble the components of PCR quickly and reproducibly. Both products have been widely sold for more than ten years. Also, I have provided testimony as a technical expert in litigation involving issues related to DNA amplification.

2. I understand that the claimed invention is directed to methods for detecting and amplifying nucleic acid molecules by using anti-foam reagents to improve fluidic handling and provide enhanced accuracy of real-time optical monitoring of amplification reaction mixtures. The claimed methods for detecting a target nucleic acid in a sample include the step of amplifying the target nucleic acid using a polymerase chain reaction carried out in the presence of an effective amount of at least one anti-foam reagent. Importantly, this anti-foam reagent does not substantially inhibit the action of the polymerase. The claimed compositions for amplifying a target nucleic acid include at least one primer molecule that hybridizes to the target nucleic acid, nucleotide triphosphates, a thermostable DNA polymerase, a detergent, and an effective amount of at least one anti-foam reagent. Again, it is important to note that this anti-foam reagent does not substantially inhibit the action of the thermostable DNA polymerase contained in the composition.

3. I have reviewed the Office Action dated April 12, 2006, and I understand that the Examiner alleges that the subject matter of claims 1, 8, 11 and 12 is disclosed in U.S. Patent No. 5,834,252 to Stemmer *et al.* ("Stemmer").

4. I further understand that the basis for the Examiner's position is that Stemmer teaches that antifoam reagents can be used in PCR reactions.

5. In my professional opinion, however, the method in respect to the use of anti-foam taught by Stemmer is deficient.

6. There is a single instance in which Stemmer discloses anti-foam agents, in the context of the inventors defining "physiological conditions" essentially as those compatible with viability of cultured yeast and mammalian cells. See column 30, lines 7 to 30. Also, at column 30, lines 14 to 16, Stemmer states that reaction conditions for polymerase chain reaction (PCR) are generally physiological conditions. However, it is my opinion that the conditions used when carrying out PCR, especially the temperatures used (*i.e.*, temperatures approaching 100 degrees Celsius), are well outside the range compatible with viability of yeast and mammalian cells. It is noted that temperature is disclosed as one of the parameters of a "physiological condition". See, Stemmer at column 30, line 8. It is thus difficult to reconcile the statement that PCR is carried out under physiological conditions (as defined by Stemmer) with recognized PCR parameters

used in the field. Further, the amplification conditions used in Examples 1 and 2 of Stemmer are in my opinion conventional and standard for PCR, but are not ones that fit the definition of physiological conditions provided by Stemmer.

7. It is also my professional opinion that the guidance as to PCR reaction conditions provided at column 30, lines 7 to 30 of Stemmer are not instructive so as to enable one of ordinary skill in the art to practice PCR as it is generally performed in the field, and in fact, would lead one away from performing PCR according to established and accepted protocols. For example, Stemmer teaches the use of 10 mM NaCl or 25 times this concentration, or any concentration in between, and 5 mM Tris–HCl or 10 times this concentration or any concentration in between. Further, Stemmer teaches that PCR can be performed at a range of 5 to 8, which represents a range of 1000 fold in hydroxide ion concentration. Stemmer further teaches that one may add a divalent cation, or not, and implies that any divalent cation is useful at any concentration; this general language is also followed for the addition of metal chelators, one or more non-ionic detergents, scintillants and membrane fractions (while not providing suitable sources thereof or useful concentrations thereof).

8. This general disclosure is inadequate to guide one of skill in the art to preparation of PCR conditions that result in the desired nucleic acid amplification. Indeed, some of the teachings, if followed, would lead one away from operative PCR conditions. For instance, addition of a divalent cation such as magnesium is recognized as necessary, not optional. Further, addition of a metal chelator may be acceptable, but not if the concentration added significantly reduced the concentration of free magnesium below the concentration needed for PCR, in which case the metal chelator would prevent PCR amplification. In my opinion, PCR requires tightly controlled reaction conditions: it is a repetitive process whereby any small variation in reaction efficiency is magnified due to the exponential increase in product formed as the PCR proceeds over dozens of cycles. The need to tightly control certain reaction conditions, notably but not exclusively, temperature, Mg ion concentration and pH, was well known to practitioners of PCR at the time Stemmer application was filed. When carrying out PCR with the goal of quantifying the amount of input target nucleic acid, the need to control the reaction conditions is accentuated. Indeed, Stemmer provided adequate PCR conditions in Examples 1 and 2. There, the concentrations of essential components are provided with specificity (for

example, the magnesium chloride concentration is provided to a certainty of 0.1 mM.) I believe that a person skilled in PCR would understand that the magnesium concentration should be 2.2 mM and not, say 2.3 mM or 2.1 mM. The contrast between the precision of the reaction conditions set out in Examples 1 and 2, which represent the customary level of precision, and that presented in column 30 in which anti-foam is mentioned is stark. As a practitioner of PCR and as one who lead a research group developing PCR products I would never use the general direction offered in the column 30 paragraph myself nor would I let those who worked for me make use of it.

9. I also understand that US patent 5,985,569 to Foxall *et al.* ("Foxall") is being cited to the Examiner in an Information Disclosure Statement filed herewith. In my opinion, Foxall discloses a method for detecting a bacterium using a nucleic acid amplification method known as strand displacement reaction (SDA). This method of amplification is not PCR. The reaction conditions for SDA are quite different from those of PCR and the amplification processes are distinct.

10. In the context of performing SDA, use of an anti-foam is mentioned by Foxall in three of the examples. In Example 7, Foxall does not specify the type of anti-foam used or teach how one skilled in the art would go about selecting an appropriate anti-foam agent. Moreover, Foxall does not offer any explanation for the inclusion of an anti-foam agent. In Example 7 Foxall describe a protocol for performing thermophilic SDA in the presence of some type of anti-foam agent; in contrast, in Example 9 primers were screened for use in thermophilic SDA, and the inventors state that antifoam was not used, again without explanation for the omission of the anti-foam. I also note that in Example 10 reaction conditions for thermophilic SDA are stated to be optimized (according to, *e.g.*, the title of the example), and anti-foam was included with the testing conditions. This inclusion strongly suggests that anti-foam was used in the optimization process. (See Foxall at column 21, lines 8 to 10.) However, following in this example is a listing of the reagents used and anti-foam is not included. Also telling, Example 10 concludes with a description of the conditions identified as producing the greatest amplification using SDA: anti-foam is absent from the listing of those reagents a skilled artisan is instructed to use in order to obtain optimal thermophilic SDA. Thus, I believe that one skilled in the art would not be led to use anti-foam in SDA since the use of this agent is not adequately described.

Recall that Example 7 suggested that one should use an unspecified type of anti-foam in an unspecified amount, but that in Example 10, in which the SDA reaction described in Example 7 was stated to have been optimized, the optimal conditions call for one not to use antifoam. Thus, a skilled practitioner in search of optimal SDA conditions would not use an anti-foam agent, based on the teachings in Foxall. If a fellow scientist were to tell me they he or she had optimized a given nucleic acid amplification reaction and had tested a number of reagents as additives, but then if the protocol provided to me from that scientist lacked a particular reagent that he said he had tested, I would infer that I should not add it based on all that he had told me.

10. I understand that US patent 5,962,273 to Durmowicz *et al.* ("Durmowicz") is also being cited to the Examiner in the Information Disclosure Statement filed herewith. In my opinion, Durmowicz, like Foxall, discloses a method for detecting a bacterium using SDA. While Foxall used DNA amplification means including SDA to identify mycobacterium, Durmowicz employed SDA to detect *Neisseria gonorrhoeae*. As stated above, I believe that the reaction conditions for SDA are quite different from those of PCR and the amplification processes are distinct.

11. Durmowicz discloses the use of an anti-foam agent; 0.015% antifoam is provided as the last compound in a listing of reaction components in Example 14. The type of antifoam to be used is not disclosed. I note that it is recognized in the field that many types and qualities of antifoam are available commercially, so that a general instruction to use 0.015% antifoam is not sufficiently descriptive for even a practitioner with substantial experience in the field of nucleic acid amplification. This point deserves some additional explanation. Anti-foam is not a defined compound in the way glycerol or DMSO are, both of which are also included in specific amounts (8% and 3%, respectively) in the reaction conditions listed in Example 14. Instructing one to include a defined compound such as glycerol or DMSO is substantially different than instructing one to include something described as anti-foam. I would understand anti-foam to mean some material used or sold for the purpose of controlling foaming. It could be a single compound or a mixture of compounds. Thus, a skilled practitioner would have to laboriously test essentially all anti-foams and combinations thereof at a range of concentrations around 0.015% in an attempt to find the anti-foam agent(s) which work optimally at a concentration of 0.015%. I would readily know that when reading a protocol calling for the use of glycerol or DMSO in a biochemistry

research publication or in a patent that glycerol or DMSO of the high purity sold by any of many reputable suppliers would work. In contrast, if I were instructed to include anti-foam, I would immediately want to know which of the many chemicals or chemical mixtures marketed to control foaming I should use. Moreover, to find the right one or ones would require extensive screening. Since anti-foams are chemically distinct, one would not expect the same concentration to work for all anti-foams, even if any anti-foam could be used. Here, the skilled artisan would have to laboriously identify which anti-foam or foams would work at a concentration of 0.015%. Further, one might find a specific anti-foam agent useful in the SDA but useful at a concentration other than 0.015%. In my experience, those in the field of biochemistry do not provide such vague instruction when disclosing experimental methods, particularly for DNA amplification techniques requiring precise guidance as to type and amount of reagents. Indeed, the reason one provides protocols is to enable others in reproducing experiments without undue experimentation (which was completed by the original scientist).

12. I conclude that the protocols disclosed in Foxall and Durmowicz would require a substantial and undue amount of experimentation to discover the right anti-foam to use for SDA, which was known to but not disclosed by Foxall or Durmowicz. Further, SDA being a very different reaction than PCR, I would be given no guidance or expectation that any anti-foam would be helpful or even compatible with PCR.

13. All statements made herein of my knowledge are true and all statements made on information and belief are believed to be true; and further, these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that such willful false statements may jeopardize the validity of the application or any document or any registration resulting therefrom.

Date: 11 Jan. 2007

Mark Berninger